

Background

Synthetic biology endeavors to rationally engineering biological function from fundamental building blocks. Such efforts have routinely been hamstrung by complications stemming from working in the complicated and often unpredictable environment of the cell. Recently, Pardee *et al.* described a novel approach to synthetic biology by freeze-drying engineered gene-networks with *in vitro* expression machinery onto paper and demonstrating functionality upon rehydration. These paper-based gene-networks exhibit transformative potential for synthetic biology and its applications, especially DoD-relevant biodetection, by offering a modality that is cheap, disposable, stable, multiplexible over targets and analyte types (RNA/small molecule/protein), rapid to design and manufacture. These freeze-dried gene networks are embeddable into paper, clothes, or other porous materials. The authors demonstrated examples of RNA and small molecule detection in multiple media, showed stability of a year at ambient conditions, and built and tested a novel Ebola assay with strain-level identification within 12 hours. In this work, we build towards future applications by exploring the robustness of these paper-based circuits to cellular lysates that might be present in real-world samples.

Relevance

Key attributes for DoD-relevant biodetection

- Cheap, disposable paper-based assays
- Stable (initial prototypes retained >90% activity after one year at room temperature)
- Rapid design and manufacture (days or less)
- Potential to be embedded into other porous materials (e.g. clothing)

Transformative synthetic biology advance

- Brings engineered circuits into a real-world-ready format
- Simplifies development for experts by offering reproducibility, natural robotic adaptation, and rapid testing
- Simplifies development for novices in the DIY community or potential bad actors

Integrates into existing platforms

- Tickets are designed to be read by the DTRA-funded handheld Volatile Organic Compound (VOC) kit reader



VOckit Reader

References:

Pardee *et al.* *Cell.* 2014
Green *et al.* *Cell.* 2014

Results: Robustness to Lysates

Expression of sfGFP via the PURExpress system was assayed with varying DNA and cellular lysate concentrations (**Figure 1-2**). Performance was highly dependent on DNA concentration and only slightly impacted by the presence of lysate. These results indicate that methods to lyse samples directly on the paper-based test tickets should not interfere with the performance of embedded synthetic gene networks designed to process inputs.

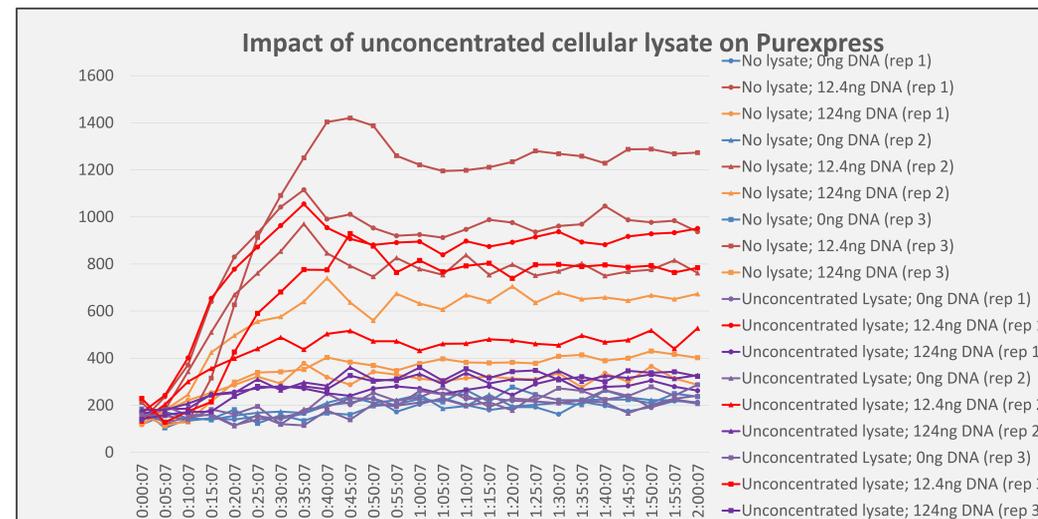


Figure 1: PURExpress *in vitro* expression mix was combined with varying amounts of sfGFP plasmid DNA (0 ng, 1.2 ng, 12.4 ng, or 124 ng) in triplicate and fluorescence measured by plate reader over 2 hours. Samples with 12.4 ng of DNA showed marked increases in fluorescence without lysate and slightly reduced increases with lysate. All other DNA concentrations showed little increase, with the single exception of 124 ng (rep 2). Note that no RNase inhibitor was used.

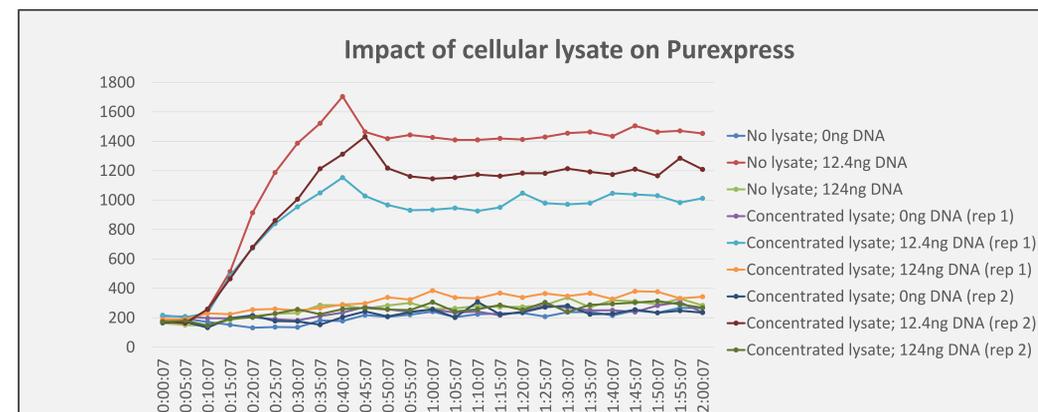


Figure 2: Methods are the same as in Figure 1, except that (a) RNase inhibitor was added, and (b) cellular lysate was concentrated by centrifugation and resuspension in 1/10th volume. Presence of the RNase inhibitor further distinguished the 12.4 ng DNA concentration from 0 ng and 124 ng. Even at 10x concentration, cellular lysate showed little inhibition on sfGFP expression.

Paper Test Tickets

Paper tickets are printed using a wax-based printer (Xerox Phaser 8560N), then baked to allow the wax to flow through to create a hydrophobic barrier between individual sample spots. The paper is then blocked using BPA. The cell-free *in vitro* expression system, RNAase inhibitor, and sfGFP plasmid DNA (or other DNA circuit). These tickets can be freeze-dried for later use by rehydration, or incubated immediately.

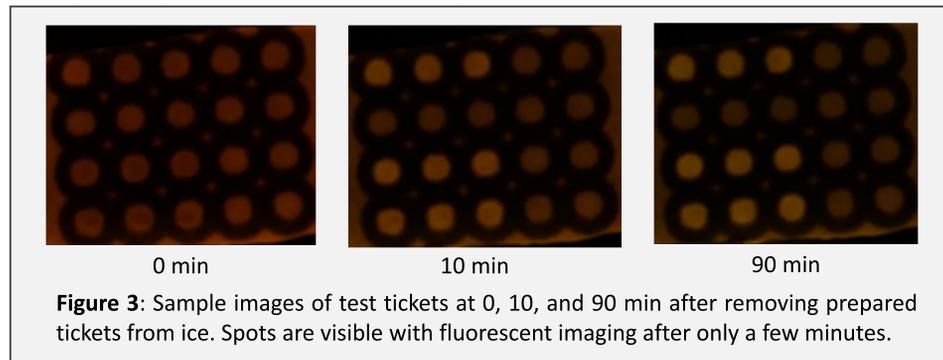


Figure 3: Sample images of test tickets at 0, 10, and 90 min after removing prepared tickets from ice. Spots are visible with fluorescent imaging after only a few minutes.

Future Directions

Near term:

- Adapt image processing software developed for VOckit reader to automate quantitation of paper spots
- Validate consistent performance of freeze-dried tickets
- Move to colorimetric reporter (LacZ)

Future projects:

- Adapt technology to DoD-relevant applications, such as detection of pathogens in lysed samples using RNA switches, RNA-aptamers for explosives detection, etc.

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